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A critical study of certain species of *Mucor**

ALFRED H. W. POVAH

(WITH PLATES 17-20)

I. INTRODUCTION

The Mucorales have been widely investigated in Europe for the past half century; consequently much has been written about them and many have been described. In this country, however, such has not been the case; for, with the exception of several papers on some of the rarer genera, Blakeslee's (1904) important publications on the sexual reproduction of the group comprise the only American contributions to a knowledge of these most interesting fungi. The Mucorales include a large number of forms, exhibiting considerable variation; in fact, the individual plants are quite plastic, so that we may obtain striking differences within the species under different external conditions, such as medium, light, temperature and moisture. It is, therefore, extremely desirable, when dealing with descriptions of species in this group, to have full data on the most important growth conditions. Thus far this fundamental fact has been under-emphasized in the work on the mucors, especially on the taxonomic side.

The object of the present work is an attempt to standardize and unify the genus *Mucor* with reference to the morphology of its individual species and their cultural reactions. The lack of a general knowledge of the physiological attributes of the many species which have hitherto been described makes it desirable to give these forms a detailed comparative study in order that some fundamental standard for their future classification may be obtained.

The investigations, upon which this paper is based, have been carried on during the years 1913, 1914, and 1915 in the Cryptogamic Laboratory of the University of Michigan under the direction of

* Contribution No. 159 from the Botanical Department of the University of Michigan.

Dr. C. H. Kauffman, whose constant interest and helpful criticism are hereby gratefully acknowledged by the writer.

II. HISTORICAL

I. CULTURE METHODS

In De Bary's *Morphologie und Physiologie der Pilze, Flechten und Myxomyceten* (1866) the application of cultural methods to *Mucor* is mentioned for the first time, in the following words: "Sie [*Mucor Mucedo*] wächst spontan und in den Culturen auf faulenden Früchten, Speisen, Zuckerlösungen und besonders üppig auf Mist." To Brefeld (1872), however, belongs the honor of having been the first to realize the necessity of employing the single spore culture method. On page 25 of the first Heft of his *Botanische Untersuchungen über Schimmelpilze*, we read the following: "Der Weg der Cultur einer einzelnen Spore unter lückenloser Verfolgung ihrer einzelnen Entwicklungsmomente, unter Vermeidung der vielen und zahlreichen Fehlerquellen, wie sie durch Invasion fremder Pilzsporen entstehen, kann allein die Basis für die Kenntniss und Klassification dieser Schimmelpilze abgeben." His method was very simple. He placed on a slide a single spore in a drop of freshly prepared horse dung decoction. The slides were observed directly under the microscope and when not in use were kept on a zinc plate covered with a bell jar standing in water to keep the air moist. He undertook a study of *Mucor Mucedo* (De Bary says "at my instigation") in order to learn whether De Bary was correct in his "apparently irregular pleomorphism" conception of the "collective so-called *Mucor Mucedo*." As a result he found that De Bary, owing to his crude culture method, had been dealing with an impure culture, having introduced *Chaetocladium* into the cycle of *Mucor Mucedo*.

G. Klebs (1898) does not go into detail with regard to his culture methods, nor does he mention a single spore culture. He used, for media, those substances which had proved the most favorable for the mold he was studying (*Sporodinia grandis* Link). These he found to be bread soaked with plum juice, slices of carrot and plum juice agar.

Oudemans and Koning (1902), making a study of the mycological flora of the soil of the Netherlands, give their technique in

detail. They used soil extract with 10 per cent. gelatin or 1.5 per cent. agar, but inasmuch as some fungi grew very slowly on these media, they added 2 per cent. of either saccharose or glucose. The following nutrient also proved very good in some cases: "wort 55, water 50," saccharose 2 per cent., gelatin 10 per cent., or agar 1.5 per cent. This medium always gave an acid reaction, but was used without neutralization. The manipulation may be outlined as follows: a fragment of humus from near the surface of the soil was introduced into a (previously sterilized) platinum crucible. About 1 c.c. of sterile water was then added, and the fragment of humus was triturated with a flamed glass rod, flattened at the end. A small amount of this infusion was transferred with a platinum loop to a test tube containing about 10 c.c. of water. The contents of the test tube were then emptied on a poured plate (Petri dish), which was inclined so as to allow the excess water to drain off. Their so-called pure cultures were made in one of the following ways: by cutting out a piece of the substratum with the organism on it; by transferring a fragment of the mycelium; or by transferring some spores to a newly poured plate.

In Hagem's (1908) method we are at once impressed with the fact that to him pure cultures from a single spore are prerequisites for any investigation. His method is as follows: with a platinum needle some spore material was transferred to a flask containing about 30 c.c. of sterile water. After a vigorous shaking to separate the spores, a few cubic centimeters of the dilution were poured into a second flask containing water. This was repeated once more and then 2 c.c. of this final dilution were poured into a Petri dish containing solid nutrient material, care being taken that the entire surface was moistened; the excess water was then poured off. After the cultures had stood for two or three days at room temperature, the cover of the Petri dish was removed and the plate was examined under the microscope for an isolated growth derived from a single spore. If such was found, it was cut out and transferred with a small amount of the substratum to a new dish. In his isolation of the soil-inhabiting forms, Hagem simply sprinkled a small amount of soil over three or four Petri dishes containing nutrient media. The one caution that he gives is that forms must be separated as soon as the

sporangia are formed; otherwise small forms are overgrown by the larger and more rapidly growing ones.

Blakeslee (1904), in his epoch-making study on the Sexual Reproduction in the Mucorineae, demonstrates his skill as a master of culture technique. In addition to the usual methods of gross and single spore culture, he devised a novel procedure for isolation of the two (plus and minus) strains. This consisted in teasing out an immature zygosporangium and placing it on a nutrient suitable for growth. After many unsuccessful attempts he discovered cases in which growth from both suspensors occurred in sufficient amount so that they (both) could be transferred to a fresh culture. In his investigations he employed many different kinds of media among which may be mentioned fruits, egg, potato, milk, urine, and bread.

It is difficult to appraise Lendner's (1908) method, which he does not present in detail. Presumably he used single spore cultures, but in discussing his methods, although he speaks of "dilution methods in use in bacteriology," he makes no mention of a single spore method.

For isolating soil fungi, Jensen (1912) mentions several methods, among which is described an iron tube for taking soil samples. This, however, proved unavailable in frozen soil. Thus he dug a trench 24 x 10 x 12 inches deep, and after removing the soil from the side of the trench with a sterile scalpel, he transferred the earth sample with the reflamed scalpel to a wide-mouthed bottle. In plating out his soil samples he either transferred particles of soil to poured plates directly, or else he made dilutions and then inoculated the poured plates with this dilution. He examined the inverted plates under the microscope, and when a germinating spore was found, he marked its location with a drop of India ink, cut it out, and transferred it to a new plate.

As Bainier (1883) and Schostakowitsch (1896, 1897, 1898) do not discuss their culture method in any great detail, it is impossible to judge whether they used a single spore as a starting point for their cultures. Schostakowitsch appears to have used bread chiefly as a substratum, while Bainier mentions having employed horse dung and also various sugar solutions in addition to bread.

Sumstine's (1910) reference to his cultural data is so frag-

mentary that it is impossible to know anything besides the substrata that he used. Among these, the only nutrient, with the exception of horse dung and meat, is bread.

2. TAXONOMIC

Inasmuch as the early history of the genus *Mucor* has been given in detail by Fischer (1892), we shall begin with the work of De Bary (1866). The latter included in *Mucor* many forms which have subsequently been shown to be distinct. For example, he attributed "an apparently irregular pleomorphism of reproductive organs" to *Mucor Mucedo*, including and confusing with it not only *Chaetocladium* but also *Thamnidium*. These somewhat startling results, as we have seen before, are to be explained by the crude culture methods in vogue in his day.

Brefeld (1872) made an important contribution to the systematic study of the Mucorales by showing that the polymorphism of previous authors did not exist. He it was who first gave us a clear conception of *Mucor Mucedo*, although he (at least in his early writings) included *Sporodinia*, *Phycomyces*, *Rhizopus*, *Chaetocladium*, and *Chaetocladium* in the genus *Mucor*, recognizing only one other genus, viz., *Pilobolus*. However, after studying *Chaetocladium* and *Piptocephalis* in detail he decided that they were both generically distinct from *Mucor*.

Van Tieghem (1873, 1875) has contributed more to our taxonomic knowledge of the Mucorales than any other person, but practically all of his work lies outside the genus *Mucor*, he having described and named only two species, *Mucor plasmaticus* and *Mucor circinelloides*. His work, therefore, in so far as the genus *Mucor* is concerned, consisted in defining the genus by removing all the closely related genera that were confused with it at that time. In his second publication (1875) we have the only reference to his idea as to the determination of the species of the genus *Mucor*. He says: "Depuis plus de trois ans que je m'occupe de cette famille, j'ai étudié et cultivé plus de trent espèces de *Mucor*, et souvent encore j'en découvre de nouvelles. Elles se répartissent en quatre sections. Le filament sporangifère demeure, en effet, simple chez les unes, tandis que chez les autres il se ramifie latéralement après avoir produit son sporange terminal. Ensuite,

suivant que, dans le premier, le filament sporangifère est doué ou non d'accroissement intercalaire, qu'il est élancé ou trapu, suivant que, dans le second, la ramification s'opère en grappe ou en cyme, chacun de ces deux groupes se partage à son tour en deux sections. La monographie de genre *Mucor* et la description détaillée des nombreuses espèces qui le constituent est donc un travail d'assez longue haleine, qui doit faire l'objet d'une publication spéciale."

Schröter (1889) did not recognize *Circinella*, *Rhizopus*, and *Spinellus* as genera, but regarded them as subgenera under *Mucor*. Under his subgenus *Eumucor*, which was the genus as we now consider it, he gave but seven species, which are reduced to five when considered from our present knowledge of the genus. In Engler and Prantl's *Die natürlichen Pflanzenfamilien* (1892) Schröter still retained his all too comprehensive genus *Mucor*, having added a fifth subgenus *Pirella*.

Saccardo (1888) included descriptions of some seventy-eight species of *Mucor*, of which less than ten are now recognized as authentic species of *Mucor*. His only separation, or attempt at such, was a division into two groups, the first of which contains those forms in which the sporangia are slightly colored at maturity; the second, those which have hyaline sporangia at maturity. Under the first division he included a subdivision containing forms with ovoid spores.

Fischer (1892) was the first to monograph the genus *Mucor*, giving twenty-one species with an analytical key. He first divided them into three sections, according to whether they were simple, monopodially or sympodially branched. These he again divided: the first section was subdivided according to the nature of the turf—whether remaining erect or soon collapsing; the second and third sections were subdivided according to their method of branching. Fischer, referring to his key, says that inasmuch as the species have not been studied thoroughly, "die folgende Zusammenstellung kann deshalb nur als eine provisorische betrachtet werden."

Schostakowitsch (1896, 1897, 1898), in his studies on the Siberian mucors, found nine species, seven of which were new to science. He found that *Mucor Mucedo* and *Mucor racemosus*, which were supposed to be of common occurrence throughout the world, grew seldom if at all in Siberia.

Although Bainier (1883*a*, 1883*b*, 1903) described sixteen new species of *Mucor*, only six of these can be identified from his fragmentary descriptions. Moreover, not more than two of the six species were completely described.

Blakeslee (1904), in dealing with the genus *Mucor*, merely designated his different forms with Roman numerals, of which I and II were homothallic forms and III to VI inclusive were species heterothallic.

Hagem (1908, 1910*b*), limiting himself to a study of the air- and soil-inhabiting forms in Norway, isolated seventeen species, of which seven were new. Although he retained *Zygorhynchus* as a genus, he did not treat *Rhizopus* in like manner, but reduced it to a "Subsectio" under *Mucor*. In his key he retains Van Tieghem's grouping into forms with simple, racemosely, and cymosely branched sporangiophores.

Lendner (1908) has satisfied a long-felt need in his publication. In the preface to his work we find that he has limited himself to a treatment of the genera which contain many recently described species. He recognizes fifty-one species under the genus *Mucor*, including *Glomerula repens* Bain., *Parasitella simplex* Bain., *Zygorhynchus Moelleri* Vuill., and *Zygorhynchus heterogamus* Vuill. under the names *Mucor Glomerula* Lendner (Bain.), *Mucor parasiticus* Bain., *Mucor Moelleri* Vuill., and *Mucor heterogamus* Vuill. respectively. He, himself, collected but eighteen species, of which seven were undescribed.

In his analytical key to the species of *Mucor*, Lendner used the arrangement of Fischer for his three large groups, viz.: (1) *Mono-Mucor* (comprising the unbranched forms), (2) *Racemo-Mucor* (branching in racemes or corymbs), (3) *Cymo-Mucor* (branching in sympodial cymes). Under the first division he includes ten species, which are arranged according to the morphological characteristics, such as height, color, size of sporangia, sporangium wall, shape of columella, spores, etc. In the second group he has twenty species, arranged in much the same fashion as in the first section. The third group is the largest, having one more species than the second. This section, for the most part, is divided in the same manner as the two previous ones, but in two places the author makes use of physiological characteristics as a

basis for separation of species. In the first place, for example, he separates *Mucor Jansseni* from the remaining species by the fact that it grows poorly on "wort gelatin" but well on bread; in the second place, he separates *Mucor Rouxii* from the last four species by the fact that on either bread or "wort gelatin" it forms a short yellow down, while the remaining species form a turf 1-3 cm. tall.

Sumstine (1910), in his thirty page publication on the North American Mucorales, throws to the winds all the work of the late contributors to the group (Fischer, Hagem, Lendner, etc.). He separates from *Mucor* (Mich.) L., as ordinarily understood, the genera *Hydrophora* Tode (which had long been discarded for the name *Mucor*) and *Calyptromyces* Karst. (a genus founded merely on the fact that the sporangiophores are branched). In speaking of this last genus, Sumstine says: "This complex group contains some forty described species but the relationship of these species is not well known. There seem to be two modes of branching, monopodial and sympodial. This branching has been made the basis for the division into two groups, Racemo-*Mucor* and Cymo-*Mucor*. . . . This division, however, is uncertain and unsatisfactory." It is, in the writer's opinion, as unreasonable to maintain two genera, one for branching and the other for simple forms, as to separate species according to their mode of branching; for it has been found, during the course of this study, that the former distinction is practically as difficult as the one to which Sumstine objects. To give an idea of the confusion caused by the arrangement which this author proposes, let us consider a few cases. His *Mucor Mucedo* L. is the common *Rhizopus nigricans* Ehrenb., *Hydrophora stercorea* Tode is *Mucor Mucedo* Fresen., *Hydrogera obliqua* (Scop.) O. Kuntze is *Pilobolus crystallinus* (Web. & Wigg.) Tode, and *Calyptromyces ramosus* Karst. is *Mucor racemosus* Fresen. It is the writer's opinion that the only new things in this paper are names for plants which we fail to recognize under their new guise.

III. TECHNIQUE

I. COLLECTION OF MATERIAL

Collecting was begun in the autumn of 1913, when various kinds of dung, including that of the horse, cow, sheep, dog, rabbit, mouse, and squirrel, were brought into the laboratory, placed in moist chambers and watched for the development of mucors. Decaying plant material was also employed, and the following gave positive results: stems of decaying apple and grape (*Pyrus Malus* and *Vitis* sp.), mushrooms (*Collybia dryophila*, *Psalliota campestris*), wood and leaves (*Pinus sylvestris* and *Phlox* sp.), *Sphagnum* sp., tomato (*Lycopersicum esculentum*), carrot (*Daucus Carota*), Brazil nuts (*Bertholletia excelsa*), puff ball (*Calvatia* sp.), and oak root with mycorrhiza. In the case of the plant material used, the material to be tested was placed in a damp chamber until there was produced a growth of mold sufficient to insure a transfer to a poured plate.

In the belief that the *Mucor* soil flora might prove interesting, the author undertook a series of isolations, which consisted in obtaining surface soil from a great variety of stations. These samples, except in the cases cited below, were all taken in the vicinity of Ann Arbor, Michigan. The following method was used: Petri dishes were wrapped in paper, sterilized, then taken to the field, where they were unwrapped and opened only long enough to fill them with soil. The cover was then replaced and sealed with a gummed label, upon which were written the collection data. At the laboratory the earth was saturated with sterile water, a drop or two of which was then transferred to a poured plate by means of a flamed platinum needle. From this culture a pure gross culture could usually be obtained, from which, in turn, a single spore culture was eventually made.

All of the forms isolated (see TABLE I), with the exception of Nos. 2, 4, 5, 6, 68, 69, 70, 71, and 72, were collected in the vicinity of Ann Arbor. The first four of these nine collections were obtained in Chippewa County, Michigan, during the summer of 1914; the rest were collected in the Adirondack Mountains, Hamilton County, New York.

For collecting in the field, it has been found most convenient

to be provided with agar slants, and to make only rough cultures. In the case of soil forms a few small pieces of soil were placed, by means of a flamed platinum wire, in the tube; while in the case of coprophilus forms a gross transfer of hyphae with spores to the slant sufficed. These cultures were purified in the laboratory. TABLE I will give the data in a clear and concise manner.

TABLE I
COLLECTION DATA OF FORMS ISOLATED

No.	Date	Substratum	Species
1.	10/10/13.	Stem of decayed grape.	<i>Mucor griseo-lilacinus</i> sp. nov.
2.	7/ 9/14.	Decaying mushroom.	<i>Mucor Ramannianus</i> Moeller
3.	10/ 7/13.	Horse dung.	<i>Mucor griseo-lilacinus</i> sp. nov.
4.	8/19/14.	Porcupine dung.	<i>Mucor hiemalis</i> Wehmer
5.	8/15/14.	Bear dung.	<i>Mucor hiemalis</i> Wehmer
6.	7/13/14.	Moldy bone in woods.	<i>Mucor hiemalis</i> Wehmer
7.	10/12/13.	Horse dung.	<i>Mucor abundans</i> sp. nov.
8.	10/12/13.	Horse dung.	<i>Pilobolus longipes</i> Van Tiegh.
9.	10/27/14.	Sandy tilled soil, surface.	<i>Mucor abundans</i> sp. nov.
10.	11/18/13.	Horse dung.	<i>Mucor saturninus</i> Hagem
11.	10/19/13.	Sheep dung.	<i>Mucor griseo-lilacinus</i> sp. nov.
12.	11/20/13.	Squirrel (?) dung.	<i>Mucor griseo-cyanus</i> Hagem
13.	11/ 5/14.	Sandy wood soil, surface.	<i>Mucor hiemalis</i> Wehmer
14.	10/28/13.	Dung.	<i>Mucor abundans</i> sp. nov.
15.	3/14/14.	Rodent dung.	<i>Mucor griseo-lilacinus</i> sp. nov.
16.	10/23/13.	Horse dung.	<i>Mucor griseo-lilacinus</i> sp. nov.
17.	2/14/14.	Decaying wood.	<i>Absidia glauca</i> Hagem
18.	10/31/13.	Stem of decaying apple.	<i>Mucor varians</i> sp. nov.
19.	11/ 4/13.	Decaying tomato.	<i>Mucor abundans</i> sp. nov.
20.	2/19/14.	Decaying pine leaf.	<i>Mucor griseo-lilacinus</i> sp. nov.
21.	10/31/13.	Stable bedding and manure.	<i>Mucor varians</i> sp. nov.
22.	2/16/14.	Horse dung.	<i>Mucor abundans</i> sp. nov.
23.	1/31/13.	<i>Psalliota campestris</i> .	<i>Mucor griseo-lilacinus</i> sp. nov.
24.	11/22/13.	Dung.	<i>Mucor aromaticus</i> sp. nov.
25.	11/ 8/13.	Horse dung.	<i>Mucor abundans</i> sp. nov.
26.	11/20/13.	Dung.	<i>Mucor griseosporus</i> sp. nov.
27.	11/18/13.	Horse dung.	<i>Helicostylum piriforme</i> Bain.
28.	11/20/13.	Dog dung.	<i>Mucor abundans</i> sp. nov.
29.	11/22/13.	Decaying <i>Calvatia</i> sp.	<i>Mucor varians</i> sp. nov.
30.	11/27/13.	Old bones.	<i>Mucor griseo-cyanus</i> Hagem
31.	11/22/13.	Rabbit dung.	<i>Mucor abundans</i> sp. nov.
32.	11/22/13.	Dung.	<i>Mucor abundans</i> sp. nov.
33.	11/20/13.	Squirrel (?) dung.	<i>Mucor abundans</i> sp. nov. and <i>Chaetocladium Brefeldii</i> Van Tiegh.
34.	11/20/13.	Dung.	<i>Mucor proliferus</i> Schostak.
35.	10/23/14.	Oak root with mycorrhiza.	<i>Mucor lamprosporus</i> Lendner
36.	10/27/14.	Surface soil, hardwoods.	<i>Mucor varians</i> sp. nov.
37.	10/24/14.	Soil below surface, hardwoods.	<i>Mucor varians</i> sp. nov.
38.	10/27/14.	Surface, tilled soil.	<i>Mucor circinelloides</i> Van Tiegh.
39.	10/27/14.	Soil below surface in corn field.	<i>Mucor circinelloides</i> Van Tiegh.
40.	11/ 5/14.	Soil below surface in U. of M. Botanical Garden.	<i>Glomerula repens</i> Bain.

TABLE I—Continued

No.	Date	Substratum	Species
41.	11/ 5/14.	Sandy surface soil at edge of thicket.	<i>Zygorhynchus Vuillemini</i> Namysl.
42.	11/ 5/14.	Soil in greenhouse.	<i>Mucor circinelloides</i> Van Tiegh.
43.	11/ 5/14.	Soil from cold frame.	<i>Mucor circinelloides</i> Van Tiegh.
44.	11/ 5/14.	Soil in greenhouse.	<i>Mucor spinescens</i> Lendner
45.	11/ 5/14.	Soil in cold frame.	<i>Mucor circinelloides</i> Van Tiegh.
46.	11/ 5/14.	Surface soil in U. of M. Botanical Garden.	<i>Mucor varians</i> sp. nov.
47.	10/17/14.	Contamination in culture.	<i>Mucor plumbeus</i> Bonord.
48.	10/17/14.	Contamination in culture.	<i>Mucor christianiensis</i> Hagem
49.	10/17/14.	On decayed leaf.	<i>Mucor sphaerosporus</i> Hagem
50.	11/ 2/14.	Contamination in culture.	<i>Mucor sphaerosporus</i> Hagem
51.	10/ 6/14.	Decayed phlox leaf.	<i>Mucor plumbeus</i> Bonord.
52.	10/ 7/13.	Horse dung.	<i>Phycomyces nitens</i> (Agardh) Kunze
53.	11/23/14.	Contamination.	<i>Thamnidium elegans</i> Link
54.	11/10/14.	Soil in greenhouse.	<i>Mucor christianiensis</i> Hagem
55.	11/ 5/14.	Soil below surface in U. of M. Botanical Garden.	<i>Cunninghamella elegans</i> Lendner
56.	11/ 5/14.	Same data as No. 55.	<i>Mucor circinelloides</i> Van Tiegh.
57.	10/27/14.	Same data as No. 39.	<i>Mucor corticolus</i> Hagem
58.	10/27/14.	Same data as No. 39.	<i>Mucor varians</i> sp. nov.
59.	11/ 5/14.	Soil in greenhouse.	<i>Mucor christianiensis</i> Hagem
60.	11/ 5/14.	Same data as No. 46.	<i>Mucor abundans</i> sp. nov.
61.	3/25/15.	<i>Sphagnum</i> with germinating seeds.	<i>Mucor plumbeus</i> Bonord.
62.	4/ 7/15.	Decayed carrot.	<i>Mucor Ramannianus</i> Moeller
63.	3/25/15.	Decaying bean testas.	<i>Glomerula repens</i> Bain.
64.	6/ 5/15.	Contamination.	<i>Rhizopus arrhizus</i> Fisch.
65.	3/21/15.	Filter paper with date seeds in damp chamber.	<i>Syncephalis</i> sp.
66.	4/ 8/15.	<i>Pilobolus</i> sp.	<i>Syncephalis cornu</i> Van Tiegh. & Le Monn.
67.	4/ 8/15.	Horse dung.	<i>Mucor hiemalis</i> Wehmer
68.	8/17/15.	Decaying <i>Collybia dryophila</i> .	<i>Mucor saturninus</i> Hagem
69.	8/31/15.	Soil in mixed woods.	<i>Absidia caerulea</i> Bain.
70.	8/31/15.	Soil in mixed woods.	<i>Mucor corticolus</i> Hagem
71.	8/31/15.	Soil in mixed woods.	<i>Absidia glauca</i> Hagem
72.	8/31/15.	Soil in mixed woods.	<i>Mucor hiemalis</i> Wehmer
73.	3/12/15.	Rabbit dung.	<i>Mucor coprophilus</i> sp. nov.
74.	3/12/15.	Woodchuck (?) dung.	<i>Mucor hiemalis</i> Wehmer
75.	1/22/16.	Decayed Brazil nut.	<i>Mucor spinescens</i> Lendner
76.	1/16/15.	Fruit of <i>Kigelia pinnata</i> (brought from Java, 7/14).	<i>Mucor corticolus</i> Hagem
77.	1/22/16.	Decayed Brazil nut.	<i>Mucor christianiensis</i> Hagem
78.	1/22/16.	Decayed Brazil nut.	<i>Mucor plumbeus</i> Bonord.
79.	1/22/16.	Decayed Brazil nut.	<i>Rhizopus nigricans</i> Ehrenb.
80.	1/22/16.	Decayed Brazil nut.	<i>Circinella spinosa</i> Van Tiegh. & Le Monn.
81.	10/19/13.	Sheep dung.	<i>Pilobolus crystallinus</i> (Wig.) Tode
82.	10/31/13.	Stable bedding and dung.	<i>Pilobolus oedipus</i> Montagne
83.	3/26/14.	<i>Sphagnum</i> with germinating castor beans.	<i>Mucor botryoides</i> Lendner
84.	8/17/14.	Decaying mushroom.	<i>Sporodinia grandis</i> Link

2. ISOLATION

As a rule little difficulty was experienced in obtaining a pure gross culture, one or two transfers to poured plates usually sufficing to eliminate obvious contaminations. In a series of closely related forms, however, there is no certainty of a pure culture, unless the latter has originated from a single spore. For practically all purposes a medium of the following composition per liter was used:

Ammonium nitrate.....	1.0 gm.
Dihydrogen potassium phosphate.....	0.5 gm.
Peptone.....	0.5 gm.
Magnesium sulphate.....	0.25 gm.
Cane sugar.....	5.0 gm.
Agar agar.....	13.0 gm.

The advantage of this medium is that all forms tried have been found to grow on it, and that cultures kept on it through the summer months (June–September) retain their vitality, at least when kept in a cool place. For stock cultures the writer has lately used a formula given him by Blakeslee, viz.:

Agar agar.....	2.0%
Peptone (Witte).....	0.1%
Dextrose.....	2.0%
Dry malt extract (Eimer & Amend).....	2.0%

This agar gives a much more luxuriant growth than the first-mentioned medium, but its use for keeping cultures longer than six weeks has not been tried by the writer.

Single spore cultures were made by a modified Kauffman's (1908) method, which is essentially an isolation of a single spore by spraying a spore dilution on a poured plate. Capillary pipettes were made, fitted with a tiny loose-fitting cotton plug immediately below the nipple. These were then sterilized in a device made as follows: two pieces of corrugated sheet asbestos, two decimeters square, were fastened together with wire in such a way that the ridges met so as to form a series of tubes between the two pieces of asbestos. The pipettes, minus the rubber nipples, were then inserted in these tubes and the apparatus was placed on a tripod over a burner and heated. Later, after the apparatus had cooled, the rubber nipples were replaced very carefully to avoid displacing the sterile air in the pipettes.

Spore dilutions were made by transferring some spore material with a platinum wire to test tubes containing about twenty cubic centimeters of sterile water each. This dilution was then sprayed on poured plates, and if the pipettes were fine enough and the spores diluted sufficiently, it was always possible to find a single spore isolated from the rest. After ten to sixteen hours, depending upon the temperature, the plates were examined to note whether germination had taken place. For this purpose the Petri dish was inverted on the stage of the microscope and examined with the low power. If a solitary germinated spore was found, the location was marked with a drop of India ink, and later the spore, together with a tiny block of agar, was cut out by a spear-pointed needle and transferred to a new poured plate. The writer has always made it a practice to watch these cultures carefully and as soon as possible to make a transfer from the edge of the growth, in order to avoid the possibility of contamination through a neighboring spore delayed in germination. This last transfer was usually made within twenty-four hours after the spore had been removed. It seems almost unnecessary to add that all the cultures used have originated from single spore cultures made in the above-described manner.

3. HERBARIUM MATERIAL

In addition to depositing living cultures in the Cryptogamic Laboratory of the University of Michigan, a series of herbarium specimens was also prepared. Inasmuch as the method employed may prove useful to others it seems desirable to include it here. For the cultures, tall lipless beakers (500 c.c. capacity) were used, with a depth of about 3 cm. of bread. If fresh bread was used, no water was added, but if dried bread was used, water was added until the medium had the humidity of the former. For a cover half of a Petri dish was fitted with a thin layer of cotton batting, and then the preparations were autoclaved for three or four hours at fifteen pounds pressure.

Pure cultures were employed for inoculation, and then the cultures were placed in the dark, in order to secure a symmetrical development. The cultures were allowed to stand, not only until the maximum growth had taken place, but until the cultures were

thoroughly dried out, in the course of which process the medium and fungus had contracted and shrunk away from the glass. Thus it was a simple matter to remove them intact. Each specimen was glued to the bottom of a cardboard box, measuring $4\frac{1}{4} \times 3\frac{1}{2} \times 1\frac{1}{4}$ inches, the cover of which was fitted with a celluloid "window" $3\frac{1}{2}$ inches long and $2\frac{1}{4}$ inches wide. It is believed that with careful handling specimens prepared in this way will retain their characters for a long time. This opinion is not without evidence to support it, as the writer has examined some of his specimens which had been kept dry for one and one half years and also Ellis & Everhart's North American Fungi, Nos. 2454 and 972. In all cases the material was in such condition that its identity could be determined with certainty.

IV. EXPERIMENTAL

The experimental work was undertaken in the hope that the results, supplemented by the morphological characters, might form a basis for the separation of species. It was desired to know whether forms morphologically similar would react similarly to the same cultural conditions, and whether forms whose morphology might lead us to believe them distinct might exhibit, or manifest, their close affinity in their cultural characteristics under the same conditions. Such has been found true only to a certain degree, but the results of the experiments are believed to be of value in the taxonomic part of this study. Consequently, experimental data have been freely used in the commentary on the individual species.

A uniform system of tabulation has been used throughout the series of experiments, in order to admit of easy comparison and also to avoid unnecessary confusion. The numbers given in the first column of TABLE I are used in all succeeding tables and throughout the work in the same way. It will be noticed in TABLES II and III that for each *Mucor* number there are two columns, the first containing Arabic, and the second Roman numerals. The former gives the height (length of sporangiophores) in millimeters, the latter the growth (judged by mass appearance), IV being excellent, III good, II fair, and I poor. It should be added that the presentation of results has been condensed on account of lack of space; hence, in the case of each medium, only the maxi-

imum growth is given irrespective of time. Furthermore, in any consideration of the numbers in the tables, it must be borne in mind that these numbers are liable to be misleading when interpreted from a standpoint of medium exclusively, because they include the inherent tendency of the species. To be specific, *Mucor griseosporus* has been found by the writer to be more plastic than other species; for example, it varies from 8 mm. to 20 mm. in height according to the medium used, while *Mucor spinescens* is always a low-growing form, heights varying only from 2 mm. to 8 mm. having been obtained.

TABLE II
HEIGHT AND GROWTH OF MUCORS WITH MISCELLANEOUS GELATIN MEDIA

Media	Mucor No. 34		Mucor No. 5		Mucor No. 4		Mucor No. 1		Mucor No. 7		Mucor No. 40		Mucor No. 16		Mucor No. 12		Mucor No. 21		Mucor No. 44	
	Ht.	G.	Ht.	G.	Ht.	G.	Ht.	G.	Ht.	G.	Ht.	G.	Ht.	G.	Ht.	G.	Ht.	G.	Ht.	G.
Levulose..	35	IV	15	III	15	III	15	III	15	III	15	III	15	III	10	II	5	II	8	II
Dextrine..	35	IV	15	III	15	III	15	III	15	III	15	III	15	III	8	II	5	II	8	II
Glucose (a)	25	IV	17	III	16	III	15	III	14	III	15	III	12	III	10	II	9	II	3	I
Glucose...	30	IV	12	III	11	III	10	III	13	III	12	III	10	III	4	II	7	II	6	I
Dung ext.	20	IV	17	III	20	III	12	III	20	III	8	II	5	II	9	II	5	II	5	I
Raulin (a)	30	IV	11	III	11	III	15	III	5	II	18	III	8	II	5	II	9	III	3	I
Maltose ..	20	IV	12	III	11	II	9	III	4	III	11	III	10	III	6	II	5	II	5	I
Lactose...	20	IV	10	II	15	III	15	III	8	III	5	II	4	II	3	II	5	II	3	I
Beef broth	36	II	12	II	14	II	11	II	9	II	7	II	9	I	9	II	8	I	5	I
Inulin....	10	III	8	II	12	II	8	II	10	II	5	II	8	II	7	II	3	II	3	I
Sucrose...	—	—	—	—	11	III	5	II	10	III	9	II	5	II	5	II	5	II	2	I
Inorg. salts	—	—	—	—	9	II	5	II	8	II	5	II	4	II	3	II	4	II	2	I
Check....	—	—	—	—	9	II	5	II	7	II	5	II	—	—	3	II	4	II	5	I

In the early experiments gelatin was used as the culture medium, but later this was replaced by agar, as the latter was found to serve the purpose equally well and, moreover, there is less possibility of its liquefaction. The following were the formulae used as given in abbreviated form in the first column of TABLE II: levulose, dextrin, glucose, maltose, lactose, inulin, and sucrose, in each case 2 per cent. of the carbohydrate with 12 per cent. gelatin; glucose (a), 5 per cent. glucose, gelatin 15 per cent. and one drop of 10 per cent. lactic acid; dung extract, fresh horse dung extracted with cold water and 15 per cent. gelatin; Raulin (a), Lendner's (1908) formula with substitution of glucose for sucrose; beef broth, 0.75 gm. Armour's beef extract, 50 gm. gelatin and 500 c.c. water; inorganic salts, 1.0 gm. KNO_3 , 0.5 gm. KH_2PO_4 ,

0.25 gm. $MgSO_4$, 0.5 gm. $CaCl_2$, 100 gm. water, and 12.0 gm. gelatin; check, 12 per cent. gelatin with distilled water.

In this experiment the cultures were made in test tubes and about 5 c.c. of the medium were used. The cultures were kept in the dark at room temperature (15–22 degrees C.). All cultures were made in duplicate, and in many cases, where there was a doubt in the mind of the writer, the experiment was repeated. The results are best obtained by consulting TABLE II, from which we see that levulose is the best medium in this series, followed by dextrin, glucose (acid), glucose, dung extract, Raulin's (acid) in the order named. Sucrose is the poorest, probably because many of the forms are unable to invert it.

TABLE III
HEIGHT AND GROWTH OF MUCORS WITH MISCELLANEOUS AGAR MEDIA

Media	<i>Mucor</i> No. 26		<i>Mucor</i> No. 34		<i>Mucor</i> No. 5		<i>Mucor</i> No. 24		<i>Mucor</i> No. 28		<i>Mucor</i> No. 6		<i>Mucor</i> No. 51	
	Ht.	G.	Ht.	G.	Ht.	G.	Ht.	G.	Ht.	G.	Ht.	G.	Ht.	G.
Rolled oats.....	70	IV	68	IV	42	IV	30	IV	40	IV	45	IV	10	IV
Glucose-asparagin agar.....	50	IV	27	IV	30	IV	18	IV	23	IV	24	IV	10	IV
Potato agar.....	65	III	55	IV	37	IV	32	IV	21	III	22	III	10	III
Corn meal.....	65	IV	60	III	35	III	20	III	30	III	25	III	5	III
Bread.....	40	III	60	III	10	III	25	III	25	III	15	III	10	III
Rice.....	50	IV	40	III	25	III	20	III	20	III	20	III	8	II
Glucose-nitrate agar.....	40	III	8	III	27	IV	13	III	17	III	19	III	13	III
Mannit-asparagin agar.....	25	II	9	III	22	III	15	III	11	III	6	II	10	III
Wheat-starch paste.....	50	III	25	II	25	III	20	III	20	II	20	III	10	II
Grapefruit.....	21	II	3	II	19	II	15	II	18	II	10	II	5	II
Sucrose-asparagin agar.....	38	II	5	I	14	II	20	II	7	II	5	II	9	II
Navy bean agar.....	27	II	33	II	13	II	20	III	9	I	8	II	5	I
Sucrose-nitrate agar.....	40	II	4	I	15	I	11	I	5	II	5	I	7	I
Mannit-nitrate agar.....	20	I	25	I	8	I	13	II	1	I	0	0	6	I
Apple agar.....	40	I	10	I	5	I	11	I	5	I	10	I	5	I
Prunes.....	1	I	1	I	10	II	1	I	1	I	—	I	1	I

In the next series of experiments various agar and complex media were used. See TABLE III. An explanation of the formulae of these media follows. Potato agar was made according to the method given by Thom (1905), and apple agar was prepared in the same way. For bean agar Thom's (1910) formula was used. For glucose-, mannit-, and sucrose-asparagin agar, and for glucose-, mannit-, and sucrose-nitrate agar, a combination of mineral salts (0.25 gm. $MgSO_4$, and 0.5 gm. KH_2PO_4) was used with 5.0 gm. of the carbohydrate, and 1.0 gm. of either

ammonium nitrate or asparagin. Rolled oats and wheat starch were both used with water in the proportion of 1 : 2 parts water by weight; corn meal was used with equal parts of water. Bread was used in the same manner as previously mentioned. Rice was used with three parts of water. Only the pulp and juice of the grapefruit were used. In the case of prunes, about one fourth of a prune was moistened with 5 c.c. of water. The results are given in TABLE III.

From this table we see that the best growth occurs on rolled oats, and that of the six best media, five are complex substances, while the sixth—glucose-asparagin agar—contains an available carbohydrate and an organic nitrogen compound. A comparison of the results obtained with glucose-asparagin agar and glucose-nitrate agar shows that the former is a more favorable medium. This is due to the form of the nitrogen offered the plant, and this nitrogen source is probably one of the important reasons why complex media are more suitable for these forms. Mannit with asparagin is better than sucrose with asparagin, but sucrose with nitrate is a better combination than mannit with nitrate. This is in accord with Jost's (1907) statement that the quality of the nitrogenous material has an influence on the nutritive value of any particular carbon compound. The growth on grapefruit was fair, while that on prunes was poor. This last result was, in all probability, due to the fact that the medium contained too high a percentage of sugar. This hypothesis is borne out by the fact that in practically all cases in which growth occurred, only a sterile mycelium developed. It should be mentioned in connection with the use of rice that, in order to determine whether there would be any difference in results with rice from different places, an experiment was set up with fifty-three numbers of mucors, using rice from different sources, purchased in Detroit and in Ann Arbor. The results showed no appreciable difference.

It seemed advisable, from the results obtained in the first two series of experiments, to select certain media and test them with a larger number of forms. Consequently a series of cultures was made with rice, bread, and grapefruit, including a large number of mucors. The height of the cultures is given in TABLE IV.

An interesting feature of the cultures on rice was the frequent

occurrence, on the surface of the medium around the edges of the culture, of a bright-colored line; this is pinkish to light orange yellow (Ridgway, 1912). Data on sixty-four numbers of *Mucor* show that thirty-four exhibited this color production, while twenty-eight numbers (Nos. 9, 10, 12, 24, 25, 26, 30, 34, 35, 38, 40, 41, 42, 44, 46, 48, 49, 50, 51, 54, 58, 59, 61, 62, 63, 68, 73, and 2) formed no color at the top of the substratum.

TABLE IV
HEIGHT OF MUCORS ON BREAD, RICE, AND GRAPEFRUIT

Species used	Rice	Bread	Grape-fruit	Species used	Rice	Bread	Grape-fruit
<i>Mucor</i> No. 1 . . .	15	15	12	<i>Mucor</i> No. 36 . . .	30	10	13
<i>Mucor</i> No. 2 . . .	2	2	0.5	<i>Mucor</i> No. 37 . . .	30	20	8
<i>Mucor</i> No. 3 . . .	20	8	11	<i>Mucor</i> No. 38 . . .	20	10	17
<i>Mucor</i> No. 4 . . .	20	30	17	<i>Mucor</i> No. 39 . . .	30	15	15
<i>Mucor</i> No. 5 . . .	25	15	19	<i>Mucor</i> No. 40 . . .	20	40	11
<i>Mucor</i> No. 6 . . .	20	15	10	<i>Mucor</i> No. 41 . . .	20	5	4
<i>Mucor</i> No. 7 . . .	30	20	12	<i>Mucor</i> No. 42 . . .	15	4	2
<i>Mucor</i> No. 9 . . .	35	8	13	<i>Mucor</i> No. 43 . . .	35	12	11
<i>Mucor</i> No. 10 . . .	10	25	14	<i>Mucor</i> No. 44 . . .	3	4	3
<i>Mucor</i> No. 11 . . .	13	10	13	<i>Mucor</i> No. 45 . . .	30	13	12
<i>Mucor</i> No. 12 . . .	20	6	10	<i>Mucor</i> No. 46 . . .	25	12	11
<i>Mucor</i> No. 13 . . .	10	—	10	<i>Mucor</i> No. 47 . . .	—	5	4
<i>Mucor</i> No. 14 . . .	30	20	13	<i>Mucor</i> No. 48 . . .	—	4	3
<i>Mucor</i> No. 15 . . .	15	7	8	<i>Mucor</i> No. 49 . . .	10	—	8
<i>Mucor</i> No. 16 . . .	15	10	15	<i>Mucor</i> No. 50 . . .	20	17	7
<i>Mucor</i> No. 18 . . .	30	12	15	<i>Mucor</i> No. 51 . . .	8	10	5
<i>Mucor</i> No. 19 . . .	35	10	10	<i>Mucor</i> No. 54 . . .	5	3	10
<i>Mucor</i> No. 20 . . .	20	8	12	<i>Mucor</i> No. 56 . . .	30	6	12
<i>Mucor</i> No. 21 . . .	30	35	11	<i>Mucor</i> No. 57 . . .	20	12	11
<i>Mucor</i> No. 22 . . .	35	10	13	<i>Mucor</i> No. 58 . . .	25	15	12
<i>Mucor</i> No. 23 . . .	10	—	9	<i>Mucor</i> No. 59 . . .	10	8	4
<i>Mucor</i> No. 24 . . .	30	25	15	<i>Mucor</i> No. 60 . . .	35	12	15
<i>Mucor</i> No. 25 . . .	25	10	7	<i>Mucor</i> No. 61 . . .	7	3	5
<i>Mucor</i> No. 26 . . .	50	40	21	<i>Mucor</i> No. 62 . . .	3	2	—
<i>Mucor</i> No. 28 . . .	25	25	18	<i>Mucor</i> No. 63 . . .	20	15	10
<i>Mucor</i> No. 29 . . .	30	22	12	<i>Mucor</i> No. 67 . . .	15	—	10
<i>Mucor</i> No. 30 . . .	15	9	12	<i>Mucor</i> No. 68 . . .	20	20	20
<i>Mucor</i> No. 31 . . .	20	20	13	<i>Mucor</i> No. 70 . . .	—	10	12
<i>Mucor</i> No. 32 . . .	25	11	15	<i>Mucor</i> No. 72 . . .	10	10	10
<i>Mucor</i> No. 33 . . .	25	15	15	<i>Mucor</i> No. 73 . . .	30	25	25
<i>Mucor</i> No. 34 . . .	40	60	3	<i>Mucor</i> No. 74 . . .	15	3	10
<i>Mucor</i> No. 35 . . .	25	12	17	<i>Mucor</i> No. 76 . . .	25	—	17

From the preceding experiments it has been shown that complex media are, on the whole, better for these plants than simple media, and as bread is a universally staple article easily obtained, as well as easy to work with, it has been used as a standard medium to grow these forms for taxonomic study.

A series of sixty-five numbers of *Mucor* (including all the species of *Mucor* collected with the exception of *Mucor botryoides*) was tested for their ability to ferment a dextrose, peptone solution (dextrose 10 per cent., peptone 0.1 per cent.) with positive results, except in the case of *Mucor Ramannianus*.

Because certain organisms have the ability to change tyrosin, by the production of an enzyme, into a dark-colored compound, often accompanied by the production of a brownish black precipitate, it was decided to use this organic compound with a series of mucors. For this experiment a solution of the following composition was employed: maltose 2 per cent., peptone 0.1 per cent., tyrosin 0.015 per cent. (1 gm. tyrosin to 6,400 c.c. of solution). The same mucors which were used in the preceding experiment were also employed for this series. The results may be given in the following brief manner. Four (Nos. 22, 36, 57, and 67) were discarded on account of bacterial contamination. In Nos. 4, 5, 6, and 72 there developed an apricot yellow (Ridgway) submerged mycelium, the color being due to yellow globules in the hyphae. The following showed a slight production of yellow color in the submerged hyphae: Nos. 1, 3, 7, 9, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 25, 28, 29, 31, 32, 33, 37, 38, 39, 42, 43, 56, 58, 60, 74, and 76. No color formation was found in Nos. 2, 10, 23, 24, 30, 34, 41, 44, 45, 46, 47, 48, 49, 50, 51, 54, 59, 61, 68, and 70. There was a marked coloration of the solution, with the formation of a dark brown precipitate, in Nos. 26 (*Mucor griseosporus*) and 73 (*Mucor coprophilus*), the color of the solution in each case being dark reddish brown. In Nos. 34, 44, 51, and 61, the solution was colored pale brown, and a dark brownish black precipitate was formed. In Nos. 40 and 63 (both *Glomerula repens*) there was a brownish tinge to the solution, and the aerial growth was distinctive in that it was pinkish buff (Ridgway).

[To be concluded.]